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COMPARATIVE FERTILITY OF GAMETES FROM SIX SPECIES OF SEA URCHINS

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Gametes from marine animals are readily fertilized *in vitro* and fertility can, therefore, be defined and investigated quantitatively, in terms of the success of gamete interaction. Sea urchin gametes are particularly suitable for such analysis because of the ease of obtaining gametes and fertilization under controllable conditions (*cf.* Tyler and Tyler, 1966a).

Several early investigators tried to determine the minimum number of sperm necessary to activate an egg (*cf.* Glasser, 1915 and Lillie, 1915). Their results were inconclusive because they evaluated sperm concentration as dilution of "dry sperm" (*i.e.*, the thick, undiluted semen exuded from the animal) rather than numbers of sperm, and defined fertility in terms of the percentage of eggs fertilized. Such research, although not truly quantitative, set precedents which have been followed in most subsequent studies (*cf.* Tyler and Tyler, 1966a, 1966b).

The objective of the research reported here was to compare the fertility of gametes from different species of sea urchins. A method was devised for determining the ratio of spermatozoons/fertilization under defined conditions. The procedure was standardized so that dilution effects and the subsequent rapid aging of sperm were minimized (*cf.* Branham, 1966). A suitable working definition of *in vitro* fertility was devised by examining the results of numerous crosses. The data were then evaluated statistically to compare the various species.

Material and Methods

Six species of regular echinoids with convenient breeding seasons are readily available around Oahu in Hawaii (Table I). Gametes were obtained from them by injecting isotonic KCl (0.53 M) into the coelomic cavity during the seasons indicated in Table I.

Injected females were inverted over a beaker so that eggs were shed directly into sea water. The eggs were washed with several changes of sea water and their concentration determined by counting the eggs in a portion of the stock suspension. Males were also inverted over a beaker and semen shed directly into sea water acidified to pH 7.0 with carbon dioxide. Under these conditions the sperm were relatively inactive and the semen accumulated as a layer on the bottom of the beaker (*cf.* Branham, 1966). Stock sperm suspensions were prepared by stirring a small volume of concentrated semen into additional acidified sea water until the desired concentration was obtained.

Sperm concentration was calculated from the optical density (O.D.) of the stock suspension determined with a Klett Summerson colorimeter (Green filter, 520–580 mμ), by multiplying O.D. by a species specific constant (see Table I).

The constants were calculated by comparing sperm concentration determined by haemocytometer counts, with the O.D., for at least one hundred sperm suspensions for each species.

Fertility titrations were carried out in a series of "wells" in plexiglas blocks ("trays") arranged so that the gametes could be viewed from below with an inverted microscope. The wells were 11 mm deep and had end areas of one cm^2. Each tray contained a single row of ten wells. Eppendorf automatic microliter pipettes with disposable tips were used to fill the wells and prepare serial dilutions of semen.

The standard procedure was to make a ten step, two-fold dilution series of the stock sperm suspension of known optical density and then add approximately 200 eggs to each well. One half ml of stock was added to 0.5 ml of sea water and one half of this dilution transferred into 0.5 ml of sea water in the next well, and so on. This procedure took less than one minute per tray. The proportion of eggs with fertilization membranes was determined by examining one hundred eggs in each well, beginning at least 20 minutes after insemination. All eggs were counted in three wells of each tray and the average number of eggs/well in that tray calculated. Sixteen trays with a total of 160 wells were used, so that four sperm suspensions could be crossed with four egg suspensions in all possible combinations. The optical density of the stock sperm suspension, the volume, the average number of eggs and the percentage of eggs fertilized in each well were recorded on IBM cards and analyzed statistically with the aid of an IBM 360 computer.

Experiments and Results

In order to evaluate the relative fertility of gametes from different sea urchins it was necessary to standardize a quantitative method for determining fertility. This has customarily been attempted by preparing a serial dilution of semen (expressed as some fraction of "dry" semen), adding eggs and determining the percentage of eggs fertilized in each sperm dilution step, or arbitrarily choosing as an end point the dilution below which less than some given percentage of fertilization occurred (*cf.* Tyler and Tyler, 1966b). This procedure was modified in the following experiments, so that the approximate number of gametes could be determined and dilution effects and sperm aging could be minimized.

Lillie (1915) observed that the sequence of mixing gametes influenced such fertility titrations. He noted that higher percentages of fertilization were obtained in greater dilutions of semen when sperm suspensions were added to eggs rather than vice versa. He demonstrated that this was the result of an effect of time after sperm dilution, which varied with the sequence used. Such aging of sperm can be controlled by keeping the sperm immobilized in acidified sea water (Branham, 1966).

Carbon dioxide, added to sea water until a pH of 7.0 was obtained, reduced the motility of sperm from the six Hawaiian species. The fertility of such immobilized sperm was compared with that of normally active sperm in the following titration experiments. "Dry" *T. gratilla* semen was diluted in sea water (pH 8.2) or CO_2 charged sea water (pH 7.0) and the optical density measured. The suspensions were serially diluted with sea water (pH 8.2) after about ten minutes and eggs

added. Sperm in CO_2 sea water were at least six, and usually eight or more, dilution steps (2^6–2^8) more "fertile" than the controls.

The repeatability of such determinations was examined in two experiments with *T. gratilla* gametes. In each experiment a pH 7.0 sperm suspension was prepared which gave about 50% fertilization after $\frac{1}{2}$ dilution in sea water. This was then transferred as quickly as possible (beginning within five minutes of initial dilution) in 0.5 ml portions into 0.5 ml of pH 8.2 sea water in each of the 160 wells of the sixteen trays. Eggs were added to each tray as it was filled (about one minute after sperm were added to the first well in each tray) and the last well received eggs 18 minutes after the first one. In both experiments the percentage of eggs fertilized in each well was the same within an 8% range and the last wells filled were not significantly different from the first ones. In two control experiments with normal sea water (pH 8.0) following the same time schedule, considerably higher percentages of eggs were activated in the first wells than in the last ones.

TABLE I

Species of sea urchins examined

Genus and species	Order and family	Season when "ripe"	Sperm concentration constants
	Order: Echinoida		
Colobocentrotus atratus	Family: Echinometridae	Sept.–July	$4.3\ 8 \times 10^7$
Echinometra mathaei	Family: Echinometridae	Sept.–May	$5.3\ 7 \times 10^7$
Echinometra oblonga	Family: Echinometridae	Sept.–May	$4.9\ 4 \times 10^7$
Heterocentrotus mammillatus	Family: Echinometridae	Sept.–May	$5.9\ 7 \times 10^7$
	Order: Temnopleuroida		
Pseudoboletia indiana	Family: Toxopneustidae	All year	$4.7\ 7 \times 10^7$
Tripneustes gratilla	Family: Toxopneustidae	All year	$3.6\ 7 \times 10^7$

These results indicated that variability due to sperm aging was important but could be controlled, and therefore, in all subsequent experiments sperm were initially diluted in acidified sea water.

Procedures of adding CO_2 immobilized sperm to eggs or eggs to sperm were compared in three experiments with *T. gratilla* gametes. The results were equivalent with both procedures but there were fewer manipulative steps involved when eggs were added to serially diluted semen so this sequence was used in subsequent titrations. The procedure is outlined in the Materials and Methods section.

Many crosses were made with this procedure and the results evaluated to produce a working definition of gamete fertility. The results of selected titrations are presented in Figure 1. The examples were selected at random from various categories of data that became apparent as the analysis progressed. *T. gratilla* gametes were on the average, about the most fertile, while *P. indiana* required the largest number of sperm/fertilization; *E. mathaei* and *E. oblonga* were chosen because they represented two species of the same genus. The remaining two graphs were chosen as representative of crosses between different species.

In the determinations in Figure 1, stock semen suspensions were prepared in CO_2 acidified sea water (pH 7.0) and their O.D. determined with a Klett Summerson colorimeter. The stock suspension was then diluted in filtered sea water

in 10 two-fold steps, beginning with a $\frac{1}{2}$ dilution so that the numbers on the *abscissa* are step numbers or the negative Log_2 of the stock sperm dilutions. One drop containing about 200 eggs was added to each 0.5 ml semen dilution. The percentage of eggs with membranes was determined after 20 minutes. Sperm/fertili-

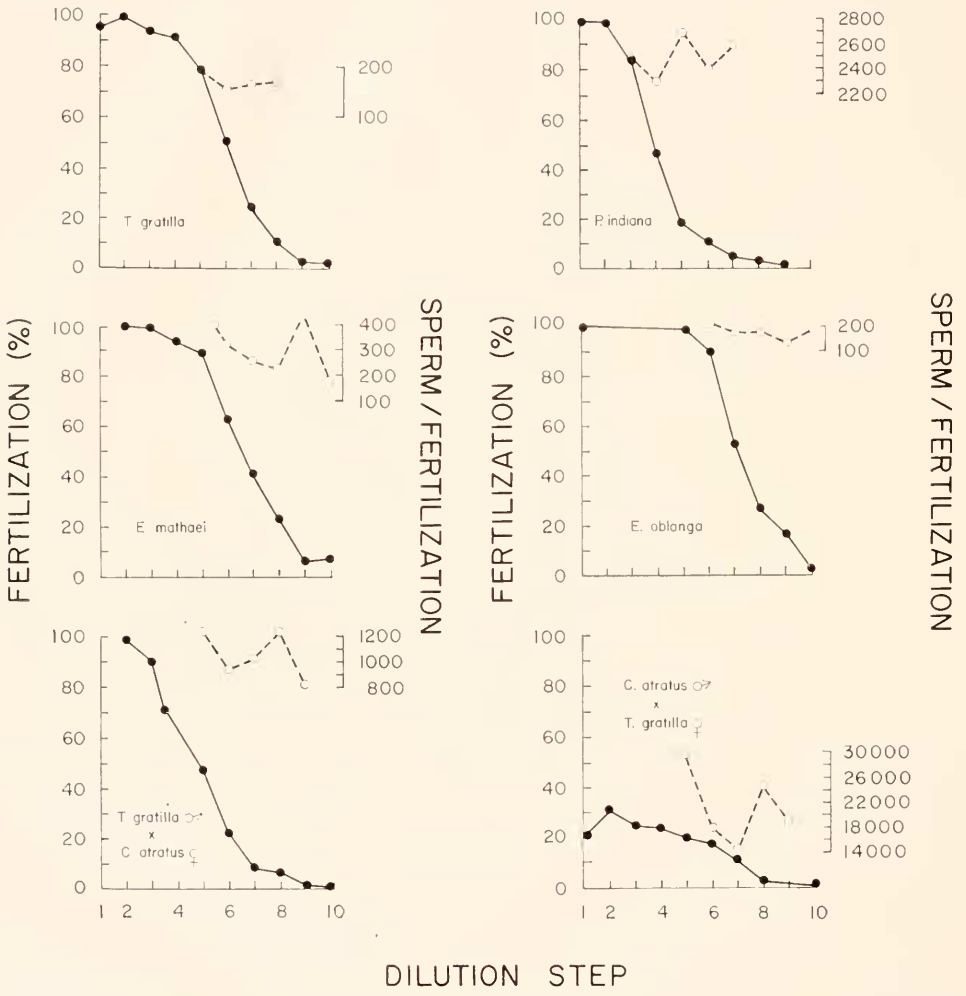

FIGURE 1. Representative titration results, comparing percentage of eggs fertilized with the number of sperm/fertilization.

zation was calculated in each well by the formula: (O.D. × sperm constant × Volume × dilution factor)/(% Fertilization × Average number of eggs per well).

In each case the first few wells contained an excess of spermatozoa and the maximum percentage of eggs were fertilized (percentage fertilized, solid line, scale on left ordinate) but in subsequent two-fold dilutions of semen the number of spermatozoa became limiting and the percentage of eggs fertilized declined. The

ratio of total number of sperm to the calculated number of eggs fertilized (dashed line, scale on the right ordinate) remained fairly constant in those wells wherein less than 91 per cent and more than 3 per cent of the eggs raised fertilization membranes. Within these limits the percentage (and also total number) of eggs fertilized declined by approximately one half for each two-fold semen dilution (in ordinary unacidified sea water, this was not so; the decline in fertilization

TABLE II

Analysis of within-species crosses

	Crosses (n)*	Average sperm/fertilization	SE†	% SE/AV	Smallest–largest sperm/fertilization
C. atratus					
	1 × 1 (16)	258	± 17	7%	178– 427
	4 × 4 (16)	116	± 14	12%	42– 235
	All (120)	631	±196	31%	14–19,010
E. mathaei					
	1 × 1 (16)	1,535	±107	7%	1,132– 2,145
	4 × (16)	658	±142	22%	254– 2,145
	All (69)	671	±109	16%	22– 5,034
E. oblonga					
	1 × 1 (16)	199	± 21	11%	135– 339
	4 × 4 (16)	265	± 47	18%	95– 670
	All (67)	356	±115	32%	21– 7,797
H. mammillatus					
	1 × 1 (16)	47	± 2	4%	32– 56
	4 × 4 (16)	64	± 8	12%	31– 139
	All (35)	112	± 15	14%	31– 398
P. indiana					
	1 × 1 (16)	2,002	± 96	5%	1,355– 2,469
	4 × 4 (16)	1,652	±406	25%	318– 5,962
	All (99)	3,542	±556	16%	218–42,766
T. gratilla					
	1 × 1 (16)	588	± 74	13%	87– 1,229
	4 × 4 (16)††	194	±100	52%	20– 1,602
	All (282)	285	± 42	15%	4– 8,048

* 1 × 1, gametes from one male and one female, subdivided into 4 semen and 4 egg suspensions which were then crossed in the 16 possible ways; 4 × 4, gametes from 4 males and 4 females crossed in 16 possible ways; *all* crosses between individuals of the same species wherein at least three wells contained 4 to 90% fertilization and there was no fertilization in the stock suspension of eggs.

† SE = Standard deviation /$\sqrt{n}$.

†† See Table III for the individual results of this series of crosses.

was more rapid, *cf.* Lillie, 1915, Figure 2). The ratio of sperm/fertilization within the prescribed limits was probably characteristic of the particular cross and was used for subsequent analysis. It or its reciprocal (fertilizations/sperm) could be used to define "gamete fertility" or "fertilizing capacity." In practice the values obtained from different wells varied somewhat, even within the chosen limits. The value characteristic of the cross was arrived at either by averaging the values that occurred within the limits or simply by using the lowest value obtained.

The data from crosses between individuals of the same species are summarized in Table II. The error inherent to the method was assayed several times for each species by making four separate egg suspensions from one female and crossing them in the sixteen possible ways with four separate sperm suspensions from a single male (1 × 1 crosses in Table II). The number of sperm per fertilization was calculated for each well and the results analyzed statistically in order to find the limits which yielded the greatest precision. The least variability, expressed as the ratio S.E./mean, in per cent, for the 16 determinations in each test resulted when the smallest value in each tray was selected for comparison with other trays. The variability was greater when average values, or the average of the three lowest values in each tray, was used for comparison, so the *smallest value in each tray was subsequently used for analysis.*

Gametes from four males and four females were also crossed in the 16 possible ways (4 × 4 crosses). The data were analyzed in the same way that the confidence limits were established, and selected examples are also presented in Table II. The examples selected for presentation were chosen at random from those

TABLE III

The number of sperm/fertilization in the sixteen simultaneous crosses between four males and four female T. gratilla *presented in Table II*

	Males			
	1	2	3	4
Females				
1	27	44	120	41
2	44	20	1,602	106
3	20	68	150	100
4	60	20	601	75

experiments wherein each of the sixteen possible crosses was successful, *i.e.,* had at least three wells with more than 3% and less than 91% of the eggs fertilized, and none of the eggs in the stock suspension were fertilized. (In some experiments the stock sperm suspension contained too many or too few sperm and the cross could not be evaluated). In most cases the amount of variability was greater than that inherent to the method determined with repeated crosses of the same gametes (1 × 1). The results of the sixteen *T. gratilla* crosses selected for analysis in Table II are displayed in Table III. They are an extreme example.

The data from all successful crosses between individuals of the same species were pooled to examine possible species differences in gamete fertility (*all,* in Table II). The average should be viewed with caution because the data were not normally distributed. In each case kurtosis and skewness were significantly greater than normal, indicating that the distributions were flatter than normal and skewed to the right (Table IV). One interpretation of the observed distributions is that some proportion of the crosses were abnormally infertile, *i.e.,* required larger numbers of sperm/fertilization than normal, and that these constituted the tail of the distribution. This possibility was examined by successively excluding an increas-

ing proportion of these highest values from each population until the distribution no longer deviated significantly from normal kurtosis and skewness ($P < 0.001$). The proportion of "abnormally" high values excluded varied for each species, as seen in Table IV.

The data from the various species were compared statistically. Such comparisons should be viewed with caution because the data deviated from a normal distribution in some cases and because the variances of the data were heterogeneous. The results of an approximate test of equality of means with heterogeneous variances (described by Sokal and Rolf, 1969) indicated that there were significant differences between the means ($P < 0.001$). The probability of difference be-

TABLE IV

Analysis of distribution of all data and selected data exclusive of "abnormally" infertile crosses†

Species	All values					Values exclusive of highest ones					
	N	Skewness	Kurtosis	Sperm/fert. AV±SE	Sperm/fert. Coe. var.††	Excluded %	N	Skewness	Kurtosis	Sperm/fert. AV±SE	Sperm/fert. Coe. var.††
C. atratus	120	6.346***	46.670***	631±196	3.40	20	96	0.442	−0.769	91± 5	0.58
E. mathaei	69	2.584***	7.636***	671±109	1.36	30	48	0.279	−1.022	232± 21	0.63
E. oblonga	67	7.619***	58.226***	356±115	2.64	15	57	0.190	−0.131	184± 10	0.42
H. mammillatus	35	1.548***	1.646*	112± 15	0.82	30	24	0.757	−0.082	60± 4	0.35
P. indiana	99	4.561***	26.077**	3542±556	1.56	25	74	0.412	−0.941	1611±116	0.62
T. gratilla	282	7.364***	66.673***	285± 42	2.48	55	127	0.415	−0.800	32± 2	0.57

† Skewness and kurtosis were computed, and their deviation from normal values evluated by t-Test, as described by Sokal and Rohlf (1969). The highest values were sequentially eliminated from the analysis, in increments of 5% of N, until both values no longer deviated significantly ($P < 0.05$) from normal.

†† Coefficient of variation = s.d./mean.

* $P < 0.050$, that statistic represents normally distributed data.

** $P < 0.01$, that statistic represents normally distributed data.

*** $P < 0.001$, that statistic represents normally distributed data.

tween means was therefore examined with a t-test matrix, in Table V, using all the data, log transformation of the data, or the values obtained after excluding the highest values, as described above.

A few crosses were made between species and the results, although too limited to be definitive, suggest a useful addition to thinking about cross fertilization (Table VI). In most cases the percentages of eggs activated were great enough to give titration curves comparable to within species crosses even though a large proportion of eggs were usually not cross activateable (see representative example in Fig. 1). The ratio of sperm/activation in these cases could be calculated as it was for within species crosses and remained fairly constant after the sperm concentration became limiting. This ratio, when considered along with the maximum proportion of eggs activatable by sperm of the other species (which varied greatly within each combination examined), and the ratio of successful cases (*i.e.* > 3% activation) to the number of trials, gave a quantitative estimation of cross reactivity that facilitated comparisons (Table VI).

Discussion

The procedure gave a rough quantitative measure of fertility. It combined traditional estimates of fertility in terms of the percentage of eggs fertilized at various semen dilutions with estimates of the number of sperm present per fertilized egg under standardized conditions. Its main fault was its large inherent standard error of about 8% of the mean values (see Table II). This error probably resulted from imprecision in determining the percentage of eggs fertilized (as shown by preliminary experiments where values varied about 8%

Table V

Probability of similarities between the mean values of sperm per fertilization of the six species, when the means were calculated from all values, the log of all values or the values remaining after the exclusion of a proportion of the highest values as indicated in Table IV

	E. mathaei	*E. oblonga*	*H. mammillatus*	*P. indiana*	*T. gratilla*
C. atratus					
All	>0.5	<0.4	<0.2	<0.001	<0.01
Log	<0.001	<0.025	<0.1	<0.001	<0.1
Less top values	<0.001	<0.001	<0.01	<0.001	<0.001
E. mathaei					
All		<0.05	<0.001	<0.001	<0.001
Log		<0.025	<0.001	<0.001	<0.001
Less top values		<0.05	<0.001	<0.001	<0.001
E. oblonga					
All			<0.02	<0.001	<0.4
Log			<0.001	<0.001	<0.001
Less top values			<0.001	<0.001	<0.001
H. mammillatus					
All				<0.001	<0.4
Log				<0.001	>0.5
Less top values				<0.001	<0.001
P. indiana					
All					<0.001
Log					<0.001
Less top values					<0.001

when repeated determinations were made with the same sperm and egg samples) and imprecision inherent in calculating the total number of fertilized eggs by multiplying the average number of eggs by the percentage fertilized. By hindsight, it would have been better to simply count all fertilized eggs in each well wherein less than 91 and more than 3% fertilization occurred, but that was not apparent until after the data were analyzed.

The variation found when gametes from four pairs of urchins were crossed simultaneously ($4 \times 4 = 16$ crosses) was usually greater than that due to the error of the method as determined from 16 simultaneous replications of the same cross (Table II). Such variation could have resulted from differences in the

TABLE VI

Evaluation of within- and between- species crosses

Females	Males *C. atratus*	*E. mathaei*	*E. oblonga*	*H. mammillatus*	*P. indiana*	*T. gratilla*
C. atratus						
Sperm/activation±SE	631±196	49,935±47,893	74,665±55,396	Not done	4,146±1,669	3,707±1,292
Range	14 to 19,010	205 to 433,064	846 to 294,806		672 to 8,657	46 to 26,328
Max. % activation		99%	86%		99%	99%
Successful/trials		9/9	5/5		5/5	26/26
E. mathaei						
Sperm/activation±SE	109,435±25,732	670±109	550,895±378,582	Not done	294,398±99,132	148,842±68,538
Range	2,311 to 229,271	22 to 5,034	974, to 4,278,392		3,322 to 963,937	6,627 to 421,074
Max. % activation	32%		40%		57%	33%
Successful/trials	10/11		11/28		9/9	6/10
E. oblonga						
Sperm/activation±SE	8,150	133,154±67,689	356±115	Not done	Not done	86,056
Range	772 to 15,528	106 to 1,261,949	21 to 7,798			416 to 171,966
Max. % activation	30%	90%				17%
Successful/trials	2/3	18/28				2/5
H. mammillatus						
Sperm/activation±SE				112±15		3,532±1,714
Range	Not done	Not done	Not done	31 to 398	Not done	448 to 9,897
Max. % activation						35%
Successful/trials						5/5
P. indiana						
Sperm/activation±SE	122,957±59,910	613,155±294,943			3,542±556	—
Range	7,756 to 320,948	12,092 to 2,147,999	Not done	Not done	218 to 42,766	—
Max. % activation	43%	44%				1%
Successful/trials	5/5	7/11				0/12
T. gratilla						
Sperm/activation±SE	76,756±28,177	94,677±27,890	23,963±22,142	14,806	—	256±42
Range	5,758 to 447,954	1,895 to 241,277	705 to 68,228		—	4 to 8,048
Max. % activation	31%	29%	60%	7%	1%	
Successful/trials	16/23	7/8	3/4	1/6	0/13	

fertilizing capacity of the sperm samples, differences in fertilizability of the egg samples or differences in the compatibility of gametes in each particular combination. Variation in sperm fertilizing capacity apparently dominated in these results, as can be seen from Table III wherein male three (and to a lesser extent four) stands out as less fertile than the others. Variations in the fertilizability of eggs (those from female one appear slightly more fertile) and particular combinations (male four and female one, male two and female three, male three and female two, and male four and female one seem to stand out) might also have had some effect.

Variation is also apparent from the large range of values obtained when all crosses within the same species are considered. Such values were not normally distributed in most cases; more fertile crosses tended to be grouped while less fertile ones were distributed over a wide range. The titration procedure usually detected all except the most infertile crosses, because the ten step sperm dilution series usually started with high enough sperm concentration to achieve at least 4% fertilization in the first three wells. The most fertile crosses were more often lost from the analysis than infertile ones, because the dilution series had not been long enough, and more than 90% fertilization occurred even at the greatest sperm dilution. Thus the ranges observed were probably less than actually occurred and the observed mean values of sperm/fertilization were probably too high because of limitation due to the method. The proportion of crosses that were "unusable" due to too high sperm concentration varied with the species; that is, crosses within the most fertile species were missed more often than those within species that generally required more sperm/fertilization. Thus, the calculated means are probably more accurate for those species with the lowest gamete fertility, than for those species that had more fertile gametes. Variation in sperm fertilizing capacity dominated in this analysis, also, in that unusually high or low values were often associated with a particular male, which tended to be consistent when crossed with various females. Seasonal and geographical effects were not apparent from these results and should be examined more specifically. It would also be of interest to compare crosses between animals from the same area with crosses between animals from different collecting sites.

There were probably species differences in mean gamete fertility. Comparisons were uncertain because of the abnormal distribution of the data but some differences are apparent from the analysis summarized in Table V. It is most probable that *P. indiana* gametes were, on the average, less fertile than those from all other species, when the data were compared in three different ways ($P < 0.001$ in all cases). On the same basis, gametes from *E. mathaei* were less fertile than those from *H. mammillatus* or *T. gratilla*. Log conversion and adjustment of the data by omitting some of the least fertile crosses (see Table V) agree to suggest that *E. mathaei* gametes were significantly different from those of *H. mammillatus*, or *T. gratilla*. Finally, the data adjusted by omitting some of the least fertile crosses suggest that gametes from *E. oblonga* might have been less fertile than those from *C. atratus*, and *T. gratilla* gametes might have been different from those of *C. atratus* and *H. mammillatus*. *H. mammillatus* gametes were probably not different from those of *C. atratus* and gametes from the two species of *Echinometra* were also probably of about the same fertility.

Some species that were alike in gamete fertility were closely related taxonomically. *C. atratus* and *H. mammillatus* are both members of the family Echinometridae of the order Echinoida (Fell, 1966). They are further alike in that both have large, flattened primary spines on the oral surface and around the periphery of the flattened test. The primary spines are limited to those areas on *C. atratus,* but in *H. mammillatus,* occur also on the aboral surface where they are large club-shaped structures with a more or less triangular cross section. *C. atratus* lives limpet-fashion on rocks high in the surf zone while *H. mammillatus* lives in crevices in the rocks, either in the surf zone or subtidally. The primary spines of both species are used to lock the animal in place on the rocks. In both species the secondary spines are short, with flat ends, and form a mosaic-like covering on the aboral surface.

The two species of *Echinometra,* are morphologically almost alike and frequently occur together (Kelso, 1970). *E. oblonga* is dark colored and most often found in the surf zone, or just subtidally, while *E. mathaei* is light colored and is usually subtidal. The two forms have been classified as subspecies of *mathaei* (Mortensen, 1943), but are probably distinct species because it was difficult to cross fertilize their gametes (only about half of the attempted crosses had more than 4% activation, and of those, most had less than 10%, see Table VI). Gametes were obtainable from both for the same season and presumably occurred together in nature.

Tripneustes and *Pseudoboletia* are also close taxonomically (Table I), but gametes from *T. gratilla* were, on the average, at least 10 or perhaps 50 times more fertile than those from *P. indiana.* Individuals of both species were found in the same subtidal locations, although *T. gratilla* were by far the most abundant. Both kinds were of about the same size, released about the same numbers of gametes following KCl injection (100×10^6 eggs, or 200×10^9 sperm from average individuals), yielded gametes the year around and were observed to spontaneously release gametes at the same time and place. Virtually no activation occurrd in the 25 attempted crosses between the two species (Table VI).

Variation in gamete fertility is of considerable importance to the study of reproduction. Such variation not only influences results of experiments but also can be of value as a way of evaluating the mechanisms of gamete interaction. Comparisons between gametes of high or low fertility, say from extreme cases within a species or between species like *T. gratilla* and *P. indiana,* could, for example, be used to elucidate the factors that characterize potentially successful gamete interactions.

Summary

(1) A technique was developed to determine the number of sperm present for each fertilization, under standardized conditions. Gametes from six species of urchins were examined.

(2) The reliability of the method was examined by analyzing 16 repeated crosses between the same set of gametes. Variability was evaluated by crossing gametes from four males and four females in the 16 possible combinations simultaneously. The results from many such crosses were pooled for statistical analysis.

(3) A wide range of values was obtained for each species and the data were not normally distributed when all values were considered.

(4) There were probably differences between the average fertility of gametes from several of the species examined while other species produced gametes of about the same average fertility as those from other taxonomically closely related species.

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